PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference PCT03-090	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. **PCT/KR2004/000119**	International filing date *(day/month/year)* **20 JANUARY 2004 (20.01.2004)**	Priority date *(day/month/year)* 20 JANUARY 2003 (20.01.2003)

International Patent Classification (IPC) or national classification and IPC

IPC7 A61K 31/60

Applicant

NEUROTECH CO., LTD. et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] (sent to the applicant and to the International Bureau) a total of 9 sheets, as follows:
      [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
20 AUGUST 2004 (20.08.2004)	17 JANUARY 2005 (17.01.2005)
Name and mailing address of the IPEA/KR Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer LEE, Mi Jeong Telephone No. 82-42-481-5601

Form PCT/IPEA/409 (cover sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International aplication No.
PCT/KR2004/000119

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   [X] This report is based on translations from the original language into the following language English, which is the language of a translation furnished for the purposes of:

   - [ ] international search (under Rules 12.3 and 23.1(b))
   - [X] publication of the international application (under Rule 12.4)
   - [ ] international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this reort as "originally filed" and are not annexed to this report):*

   [ ] the international application as originally filed/furnished

   [X] the description:
   pages 2 3 5-7 12-31 as originally filed/furnished
   pages* 1, 4, 8-11 received by this Authority on 20/08/2004
   pages* received by this Authority on

   [X] the claims:
   pages as originally filed/furnished
   pages* as amended (together with any statment) under Article 19
   pages* 32-34 received by this Authority on 20/08/2004
   pages* received by this Authority on

   [ ] the drawings:
   pages as originally filed/furnished
   pages* received by this Authority on
   pages* received by this Authority on

   [ ] the sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [X] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [X] the claims, Nos. 11-13
   - [ ] the drawings, sheets
   - [ ] the sequence listing *(specify)* :
   - [ ] any table(s) related to sequence listing *(specify)* :

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets
   - [ ] the sequence listing *(specify)*:
   - [ ] any table(s) related to sequence listing *(specify)* :

** If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International aplication No.
PCT/KR2004/000119

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims		YES
	Claims	1 - 10	NO
Inventive step (IS)	Claims		YES
	Claims	1 - 10	NO
Industrial applicability (IA)	Claims	6 - 10	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are referred to in this report.

D1: J. Cell Biol. Vol. 159(5), pp. 821-831 (2002)
D2: Neurobiol. Dis. Vol. 9, pp. 24-37 (2002)
D3: J. Neurochem. Vol. 82(4), pp. 894-902 (2002)
D4: Neurobiol. Dis. Vol. 7, pp. 251-259 (2000)

Neurotrophins are known to have dual actions: one is to protect nerve cells by inhibiting apoptosis of the nerve cells, and the other is to potentiate or cause neuronal necrosis at the same time.

Claims 1-5 of the present invention relate to a method for treatment or prevention of neuronal death, by concurrent administration of neurotrophins (NGF, BDNF, NT-3, NT-4/5 etc.) and anti-oxidants(NADPH oxidase inhibitors such as AEBSF, vitamin E analogues such as trolox, BAS etc.). Claims 6-10 of the present invention relate to a composition for treating or preventing neuronal death comprising the said neurotrophins and the said anti-oxidants.

1. Novelty and Inventive Step

D1 discloses that the selective inhibitors of NADPH oxidase prevent BDNF-induced reactive oxygen species (ROS) production and neuronal death without blocking antiapoptosis action of BDNF. D2 describes that the addition of NT-3 to primary cortical cell cultures enhances the production of reactive oxygen species and exacerbated neuronal death caused by oxygen-glucose deprivation, and that coincubation with the oxygen free radical chelator, trolox, diminishes the potentiation of neuronal death. D3 discloses that neuronal death induced by BDNF or NT-4/5 is significantly attenuated by AEBSF. D4 also discloses that neuronal death following exposure to Fe2+ is significantly increased by concurrent addition of NT-4/5 and suppressed by addition of trolox.

As mentioned above, the technical features of combination therapy comprising neurotrophins and antioxidants in claims 1-10 of the present invention are disclosed in D1-D4. Therefore, the novelty of claims 1-10 can be negated by D1-D4.
(Continued on Supplemental Sheet.)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Box V.

Since the novelty of claims 1-10 cannot be acknowledged, the inventive step of claims 1-10 cannot be acknowledged, either. Even when the novelty of claims 1-10 can be acknowledged, the inventive step of claims 1-10 cannot be acknowledged, as long as there is no surprising effect in combining neurotrophins and antioxidants [Article 33(2) and 33(3) PCT].

2. Industrial Applicability

The subject-matter of claims 6-10 appears to be industrially applicable.
The subject-matter of claims 1-5 relates to a method of therapeutic treatment. Concerning the assessment of the industrial applicability of the subject-matter relating to therapeutic applications, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims [Article 33(4) PCT].

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METHOD AND COMPOSITION FOR TREATING NEURONAL DEATH

TECHNICAL FIELD

The present invention relates to a pharmacological composition for prevention of neuronal necrosis induced by neurotrophins, more particularily, to a method for prevention of neurotrophin-induced neuronal death by anti-oxidants and synergetic effects of neurotrophins and anti-oxidants for enhanced promotion of neuronal survival.

BACKGROUND ART

Survival of central and peripheral neurons largely depends upon contact with neurotrophins that are released from their target cells (Levi-Montalcini, 1987, *EMBO J.*, 6, 1145-1154; Barde, 1994, *Prog. Clin. Biol. Res.*, 390, 45-56). The neurotrophic effect of neurotrophins is initiated through binding to TrkA, TrkB, or TrkC, the high affinity neurotrophin receptors with tyrosine kinase activity (Patapoutian and Reichardt, 2001, *Curr. Opin. Neurobiol.*, 11, 272-280; Kaplan and Miller, 2000, *Curr. Opin. Neurobiol.*, 10, 381-391). The Trk tyrosine kinases activate the small GTP-binding protein Ras, PI-3K, and PLC□, which play an important role in survival of a variety of neurons including cerebellar granule, cortical, hippocampal, sympathetic, and sensory neurons (Borasio *et al.*, 1993, *J. Cell. Biol.*, 121, 665-672; Stephens *et al.*, 1994, *Neuron*, 12, 691-705; Yao and Cooper, 1995, *Science.*, 267, 2003-2006; Nobes *et al.*, 1996, *Neuroscience.*, 70, 1067-1079; Nonomura *et al.*, 1996, *Brain Res Dev Brain Res.*, 97, 42-50; Alcantara *et al.*, 1997, *J Neurosci.*, 17(10), 3623-3633; Hetman *et al.*, 1999, *J*

The present invention provides a method for preventing neurotrophin-induced neuronal cell necrosis with administration of anti-oxidants.

The present invention provides a method for preventing neuronal apoptosis and necrosis at the same time with concurrent administration of neurotrophins and anti-oxidants.

The present invention provides a method for preventing neurotrophin-induced neuronal cell necrosis with administration of benzylaminosalicylic acid derivatives.

The present invention provides a method for preventing neuronal apoptosis and necrosis with concurrent administration of neurotrophins and benzylaminosalicylic acid derivatives.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings.

Fig. 1 is a graph showing neurotrophins-induced neuronal necrosis in cortical cell cultures.

A : treatment with BDNF B : treatment with NT-3 C : treatment with NT-4/5

Fig. 2 is graph showing neuronal necrosis in brain sections stained with hematoxylin-eosin (H&E) at 2 day after intrastriatal injections of saline or BDNF.

A : Bright field photomicrogrphs of brain sections stained with H&E after intrastriatal injections of saline

F : The oxidized DCF in cortical neurons following exposure to BDNF plus DPI

Fig. 16 is a graph showing analysis of neuronal death by measurement of LDH efflux into the bathing medium in cortical neurons following exposure to BDNF, BDNF + DPI, BDNF + AEBSF or BDNF + 2-Hydroxy-TTBA.

Fig. 17 is a graph showing analysis of neuronal apoptosis in neuron-rich cortical cell cultures following exposure to serum deprivation, alone or in the presence of BDNF, BDNF plus DPI, DPI, BDNF plus trolox, or trolox.

DETAILED DESCRIPTION OF THE INVENTION

The detailed description of the present invention is as follows:

The present invention provides a method for preventing neurotrophin- induced necrosis with administration of drugs that block oxidative stress.

Anti-oxidants in the present invention can be chosen from NADPH oxidase inhibitors, vitamin E, vitamin E analogue or benzylaminosalicylic acid derivatives. NADPH oxidase inhibitors can be selected from diphenylene iodonium (DPI) or 4-(2-amonoethyl)-benzensulfonyl fluoride (AEBSF). Vitamin E analogue is trolox, a membrane-permeable form of vitamin E. Benzylaminosalicylic acid derivatives can be selected from BAS(5-benzylaminosalicylic acid), TBAS(5-(4-trifluoromethylbenzyl) aminosalicylic acid), NBAS(5-(4-nitrobenzyl) aminosalicylic acid), CBAS(5-(4-chlorobenzyl) aminosalicylic acid), MBAS(5-(4-methoxybenzyl) aminosalicylic acid), FBAS(5-(4-fluorobenxyl) aminosalicylic acid), and 2-hydroxy-TTBA(2-Hydroxy-5-(2,3,5,6-tetrafluoro-4trifluoromethyl- benzylamino)-benzoic acid.

Neurotrophins in the present invention can be selected from nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), and BNDF is more preferred.

BDNF causes neuronal cell necrosis by inducing expression and activation of NADPH oxidase and subsequent production of reactive oxygen species (ROS).

Administration of DPI or AEBSF prevents BDNF-induced neuronal cell necrosis by inhibiting NADPH oxidase and ROS production. Vitamin E or it analogue trolox prevents BDNF-induced neuronal death by blocking ROS production. Benzylaminosalicylic acid derivatives- BAS, TBAS, NBAS, CBAS, MBAS, FBAS, and 2-Hydroxy-TTBA – block free radical neurotoxicity as anti-oxidants (WO 01/79153), which prevents BDNF-induced neuronal death.

Thus, anti-oxidants in the present invention can prevent neurotrophin-induced neuronal cell necrosis.

The present invention provides a method for preventing neuronal apoptosis and necrosis with concurrent administration of neurotrophins and anti-oxidants.

Anti-oxidants in the present invention can be chosen from NADPH oxidase inhibitors, vitamin E, vitamin E analogue or benzylaminosalicylic acid derivatives. NADPH oxidase inhibitors can be selected from DPI or AEBSF. Vitamin E analogue is preferably trolox, a membrane-permeable form of vitamin E. Benzylaminosalicylic acid derivatives can be selected from BAS, TBAS, NBAS, CBAS, MBAS, FBAS, and 2-Hydroxy-TTBA.

Neurotrophins in the present invention can be selected from brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5, and BDNF is more

preferred.

While neurotrophins promote neuronal survival by blocking apoptosis but can cause neuronal necrosis through production of ROS. The latter can be blocked by administration of anti-oxidants. Interestingly, concurrent administration of anti-oxidants greatly enhances effects of neurotrophins promoting neuronal survival by blocking the pro-necrotic actions of neurotrophins.

Thus, co-administration of neurotrophins and anti-oxidants can be applied to prevent apoptosis and necrosis in hypoxic-ischemic injury (Holtzman *et al.*, 1996, *Ann. Neurol.*, 39(1), 114-122; Ferrer *et al.*, 2001, *Acta neuropathol.(Berl.)*, 101(3), 229-38), chronic spinal cord injury (Jin *et al.*, 2002, *Exp. Neurol.*, 177(1), 265-75), Alzheimer's disease (Siegel and Chauhan, 2000, *Brain Res. Brain Res. Rev.*, 33, 2-3), Parkinson's disease (Bradford *et al.*, 1999, *Adv. Neruol.* 80, 19025), ALS (Louvel *et al.*, 1997, *Trends. Pharmacol. Sci.*, 18(6), 196-203), Huntington's disease (Perez-Navarro *et al.*, 2000, *J. Neurochem.*, 75(5),2190-9), glaucoma (Ko *et al.*, 2000, *Invest. Ophthalmol. Vis. Sci.*, 41(10), 2967-71) or retinal detachment (Lewis *et al.*, 1999, *Invest. Ophthalmol. Vis. Sci.*, 40(7), 1530-1544).

The present invention provides an inhibitor for preventing neurotrophin-induced neuronal cell necrosis and thus enhancing survival effects of neurotrophins with administration of benzylaminosalicylic acid derivatives.

A drug containing benzylaminosalicylic acid derivatives as an effective component can be applied to prevent ROS production and neuronal cell necrosis by neurotrophins. Benzylaminosalicylic acid derivatives in the present invention can be selected from BAS, TBAS, NBAS, CBAS, MBAS, FBAS, and 2-Hydroxy-TTBA.

Neurotrophins in the present invention can be selected from brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5, and BDNF is more preferred.

The composition of the present invention can be treated by oral administration, intravenous injection or non-oral administration, and treated by various forms such as tablet, capsule, powder, grain, sterilized solution, suspension or suppository for rectal administration. Major effective elements of the composition can be made as a solid tablet using pharmaceutical carriers, for example common tablet element such as corn dextrin, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, decalcium phosphate or gums, and additional pharmaceutical diluted solution. Tablets or pillets of the pharmaceutical composition in the present invention can be manufactured for sustained release dosage form as facilitated forms for administration using well-known coating method etc. in the appropriate industry. For example, tablets or pillets can be composed with inner and outer administrative elements. The inner administrative elements of tablets or pillets can be manufactured as wrapped with outer administrative elements. Liquid forms of the composition in the present invention manufactured for oral administration or the injection include solution, appropriately flavored syrup, water-soluble suspension, water-insoluble suspension, emulsion made by edible oil such as cotton oil, sesame oil, coconut oil, or peanut oil, elixir, and similar pharmaceutical carriers. Tragacanth gum, acacia, alginic acid sodium salt, dextran, sodium carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, or synthesized or natural gums like gelatin etc can be used as appropriated aid to dispersion or suspension in making water-soluble suspension.

Quantity of medication can be determined by several related factors such as

What is claimed is:

1. A method for treatment or prevention of neuronal death, by concurrent administration of neurotrophin and anti-oxidant.

2. The method according to claim 1, wherein the neurotrophin is selected from the group consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4/5.

3. The method according to claim 1, wherein the anti-oxidant is selected from the group consisting of NADPH oxidase inhibitors, vitamin E, vitamin E analogue and benzylaminosalicylic acid derivatives.

4. The method according to claim 3, wherein the benzylaminosalicylic acid derivative is at least one selected from the group consisting of BAS(5-benzylaminosalicylic acid), TBAS(5-(4-trifluoromethylbenzyl) aminosalicylic acid), NBAS(5-(4-nitrobenzyl) aminosalicylic acid), CBAS(5-(4-chlorobenzyl) aminosalicylic acid), MBAS(5-(4-methoxybenzyl) aminosalicylic acid), FBAS(5-(4-fluorobenxyl) aminosalicylic acid) and 2-hydroxy-TTBA(2-Hydroxy-5-(2,3,5,6-tetrafluoro-4-trifluoromethyl- benzylamino)-benzoic acid).

5. The method according to claim 1, wherein the method is used for therapy or prophylaxis of Hypoxic-ischemic injury, Chronic spinal cord injury, Alzheimer's

disease, Parkinson's disease, Amyotrophic lateral sclerosis, Huntington's disease, Glaucoma or Retinal detachment.

6. A composition for treating or preventing neuronal death, comprising a therapeutically effective amount of neurotrophin and anti-oxidant.

7. The composition according to claim 6, wherein the neurotrophin is selected from the group consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4/5.

8. The composition according to claim 6, wherein the anti-oxidant is selected from the group consisting of NADPH oxidase inhibitors, vitamin E, vitamin E analogue and benzylaminosalicylic acid derivatives.

9. The composition according to claim 8, wherein the benzylaminosalicylic acid is at least one selected from the group consisting of BAS(5-benzylaminosalicylic acid), TBAS(5-(4-trifluoromethylbenzyl) aminosalicylic acid), NBAS(5-(4-nitrobenzyl) aminosalicylic acid), CBAS(5-(4-chlorobenzyl) aminosalicylic acid), MBAS(5-(4-methoxybenzyl) aminosalicylic acid), FBAS(5-(4-fluorobenxyl) aminosalicylic acid) and 2-hydroxy-TTBA(2-Hydroxy-5-(2,3,5,6-tetrafluoro-4-trifluoromethyl-benzylamino)-benzoic acid).

10. The composition according to claim 6, wherein the method is used for

therapy or prophylaxis of Hypoxic-ischemic injury, Chronic spinal cord injury, Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Huntington's disease, Glaucoma or Retinal detachment.

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